UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/158,272	09/22/98	DIAS V	10806-64

HM12/1030

DINSMORE & SHOHL
1900 CHEMED CENTER
255 EAST FIFTH STREET
CINCINNATI OH 45202

EXAMINER	
WOITACH, J	
ART UNIT	**PAPER NUMBER**
1632	16

DATE MAILED: 10/30/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/158,272	Applicant(s) DIAS ET AL.	
	Examiner Joseph Woitach	Art Unit 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 January 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *27,28,31-33,35-50 and 52-60* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27,28,31-33,35-50 and 52-60* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *22 September 1998* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

File

DETAILED ACTION

This application filed September 22, 1998, claims benefit of provisional application 60/062,994, filed October 23, 1997, and claims priority to foreign application 9703663-6 filed October 8, 1997 in Sweden.

Applicants After Final amendment filed January 22, paper number 13, has been received and entered. Claims 29, 30 and 51 have been canceled. Claims 27, 28, 33, 38-40, 52, 53, 55 and 60 have been amended. Claims 27, 28, 31-33, 35-50 and 52-60 are pending and currently under examination.

In view of Applicants amendments and arguments presented in the after final amendment, PROSECUTION IS HEREBY REOPENED. A new grounds of rejections are set forth below.

Specification

The disclosure is objected to because of the following informalities: The specification contains sequence listings which do not have a SEQ ID NO: (see for example page 5; lines 3-4 and page 7; lines 18-19). The nucleotide sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998,

see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

Appropriate correction is required.

The absence of proper sequence listing did not preclude the examination on the merits however, **for a complete response to this office action, applicant must submit the required material for sequence compliance.**

Claim Objections

The claims are objected to for the following informalities:

It is noted that Applicants had elected group I, claims 27, 28, 31-50 and 52, drawn to drawn to methods of genetic modification in transgenic animals (see Applicants election filed December 13, 1999, paper number 6. Originally, claims recited a 'method for transgenic work in eukaryotic cells' however this portion of the preamble has been deleted, and presently, the claims recite a 'method for mediating intramolecular recombination ... in eukaryotic cells'. The claims as presently amended are drawn to mediating recombination in eukaryotic cells for any reason, including gene therapy which was non-elected group IV, and the means for delivery of the recombinase and target sequences are not limited to methods commonly used in generating transgenic animals. Though by election of Group I, Applicants are limited by estoppel to methods of generating transgenic animals comprising the use of beta recombinase and the target

six site sequences, it is suggested that the preamble be amended to more clearly indicate the elected invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 27, 28, 31-33, 35-50 and 52-60 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of mediating intramolecular recombination between two *six* sites in a mouse, comprising providing a transgenic mouse whose genome contains two *six* sites target sequences in a gene of interest in said genome, administering beta recombinase, wherein the administration of beta recombinase results in the recombination between the two *six* sites, does not reasonably provide enablement for the practice in all animals. Enablement is considered in view of the Wands factors (MPEP 2164.01(a)).

Claims 27, 28, 31-33, 35-50 and 52-60 are drawn to methods of using beta recombinase in the generation of transgenic animals. Beta recombinase is a site specific recombinase originally isolated from a prokaryotic system. The art of record and the specification teach that beta recombinase can mediate the an intramolecular recombination event between two target inverted repeats termed *six* sites. Depending on the orientation of the *six* sites, the recombination and resolution of the event results in either deletion or inversion of the sequence between the two

six sites. Further, the specification teaches that the beta recombinase is capable of mediating the recombinant event in eukaryotic cells, however the specification and the art of record teaches that cofactors, bacterial Hbsu and HU, or mammalian HMG, are strictly required for beta recombinase mediated recombination. In view of the teachings of the specification, the use of the claimed methodology is for the conditional knock-out or knock-in of a gene of interest in the creation of transgenic animals.

The specification teaches specifically that beta recombinase can mediate a recombination event between two *six* sites in eukaryotic in the presence of the proper cofactor(s), however a necessary feature of the invention is the requirement of the two *six* sites present in the DNA. Presently, the only methodology known for the targeted introduction of sequences into a gene of interest, and the subsequent generation of a transgenic animal is through the use of embryonic stem cells. The basis of this rejection focuses on the failure of the instant specification and the art to teach embryonic stem cells for the generation of a transgenic animal other than that for the mouse. The specification is silent with respect to other methodology for introducing *six* sites into a gene of interest besides through homologous recombination in embryonic stem cells, and presently, to produce an animal in which the desired gene has been disrupted through homologous recombination, embryonic stem (ES) cells are necessary. Currently, only ES cells for the mouse are available (reviewed in Seamark and Moreadith *et al.*). There is no guidance in the instant specification, nor the art of record, for the use of appropriate vectors, the specific promoter sequences or cloning details for breadth of any eukaryotic cell in the context of a

transgenic animal, nor operable methods to create any transgenic animal besides the transgenic mouse. The present application has defined a novel function for beta recombinase in eukaryotic cells, and proposes the use of the beta recombinase in methodology previously described for different but related recombination systems such as CRE/lox and FLP/frt. However, neither the instant specification, nor the art of record, has resolved the many complexities involved in targeting inverted repeat sequences, such as the *six* site sequences, to the gene of interest through homologous recombination in all animals without the use of embryonic stem cells.

In addition, the claims require that beta recombinase be provided to the eukaryotic cell. The means of or route of administration for providing the beta recombinase is not specifically recited, however at least one means contemplated is through the expression as a transgene. It is noted that the physiological art in general is acknowledged to be unpredictable (MPEP 2164.03). This is particularly true in the art of transgenic animals with respect to transgene behavior. Without evidence to the contrary, transgene expression in different species of transgenic animals is not consistent and varies according to the particular host species. This observation is specifically supported by Hammer *et al.* report the production of transgenic mice, sheep and pigs; however, only transgenic mice exhibited an increase in growth due to the expression for the gene encoding human growth hormone (pages 276-277, Subsection: Effect of Foreign GH on Growth). The observation is further supported by Mullins *et al.* who report on transgenesis in the rat and larger mammals. Mullins *et al.* state that "a given construct may react very differently from one species to another" (page S39, Summary). Wall *et al.* further report that "transgene expression

and the physiological consequences of transgene products in livestock are not always predicted in transgenic mouse studies" (page 2215, first paragraph). In the instant case, there is no clear teaching on the level of expression of beta recombinase needed to mediate the recombination event between two *six* sites in a eukaryotic cell. Since the applicants have not disclosed all the nucleic acids encompassed by the claims, there is no way to predict efficiency nor expression of a transgene.

Finally, the specification and the art of record clearly teach that cofactors are necessary for beta recombinase to mediate a recombinant event. As detailed in the specification and the previous office action, the necessity of HMG1 in mammalian cells or HU and/or Hbsu from a prokaryotic source is absolute for the resolution of the recombination event catalyzed by beta recombinase. As taught by Alonso *et al.*, the Hbsu is required for the resolution and DNA inversion mediated by beta recombinase (JBC, page 938). Substitution of HU from *E. coli* or of mammalian HMG1 for Hbsu functions *in vitro* as a chromatin associated protein affecting recombination (Mol Bio, page 471), however in the absence of either of these three factors, recombination does not occur (JBC, page 2943). While HMG1 may exist in other mammalian sources, the specification and the art of record is silent to whether HMG1 or a functional homolog exist in species other than mammals. Further, while Hbsu, HU or HMG1 could theoretically be supplementally supplied, the instant methods do not recite such an active step. In addition, while the specification and art teaches that these cofactors are required for beta recombinase activity, the specification is silent on the amounts or levels of expression if supplied

as a transgene of these cofactors which are required to effectively act as cofactors. The instant specification and the art of record teaches that specific chromatin cofactors are required for beta recombinase activity, however it fails to provide a nexus with the necessary guidance which enables the artisan to supply these cofactors in effective amounts resulting in beta recombinase activity in transgenic animals.

While the methodology to create transgenic mice is routine, the creation of any transgenic animal is not. In particular, no ES cell for animals other than mice exists to date, so the creation of animals which depend on homologous recombination are not enabled in the art. Further, while methods for the introduction of a gene are routine, the expression of the gene and resulting phenotype of the animal is not. Without an actual reduction to practice, it is possible to predict that introduction of a transgene or an alteration to a gene would result a predictable phenotype or even in a viable animal.

In view of the lack of guidance, working examples, breadth of the claims, the level of skill in the art and the state of the art at the time of the claimed invention was made, it would have required one of skill in the art undue experimentation to practice the invention as claimed.

Claims 27, 28, 32, 53 and 55 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed."Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

The instant claims encompass use of 'specific target sequences', however the specification and the art of record clearly teach that beta recombinase can use only the polynucleotide sequences set forth as the *six* site sequence. Presently, in order to practice the invention as claimed the artisan must be in possession of the appropriate target sequence for beta recombinase to bind and affect recombination. The specification describes methods of using beta recombinase and the requirement of target sequences for targeting recombination, however, the only target sequence disclosed is the *six* site. The specification fails to provide any other sequence besides the *six* site sequence as a target sequence for beta recombinase. The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants effective filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics (as it relates to the claimed invention as a whole) such that a person skilled in the art would recognize that the inventor had possession of

the claimed invention. *Pfaff v. Wells Electronics, Inc.*, 48 USPQ2d 1641, 1646 (1998). In the instant case, the claimed embodiment of 'specific target sequences' needed to make and use the invention as claimed lack a written description. The specification fails to describe any polynucleotide encompassed in the claims with particularity to indicate that Applicants had possession of the claimed invention beyond the target *six* site sequence. Further, the specification fails to describe methods to establish any other sequence besides the *six* site. The written description of a claim is evaluated on the basis of the claimed invention as a whole. Case law established that the requirement for written description relates to the subject matter defined by the claims. *In re Wright*, 9 USPQ2d 1649 (Fed. Cir. 1989). To this end, while *six* site sequences meet the written description, no other specific sequence which meets the limitation of functioning as a target site for beta recombinase is adequately described or shown to exist. Thus, the specification fails to demonstrate possession of the invention as claimed. The skilled artisan cannot envision the detailed structure of the claimed target sequences except the *six* site sequence, and thus, conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Case law has established that one cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants effective filing date. Adequate written description requires more than a mere statement that it is part of the invention and reference to a

potential method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483. In *Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, the polynucleotide sequences needed to make and use the claimed invention do meet the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 27, 28, 31-33, 35-50 and 52-60 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically:

Claim 27, 28, 53 and 55 are unclear in the recitation of 'its specific target sequences' because the specification and the art of record teach that only the *six* site sequence functions as a target sequence (page 2), and it is unclear what other sequences may function as additional target sequences. In addition, claim 32 is unclear in the recitation of 'site-specific intramolecular

recombination' because it is unclear to what specific sites the claim refers. It is unclear if the claim refers to the *six* sites or other target sequences.

Claim 33 is vague and unclear in the recitation of 'two related genes' because it is unclear how the gene are related one to the other. The claim refers to 'different DNA sequences', however there in no indication in how the sequences are related to a gene. In addition, neither independent claim 27 nor intervening claims state that multiple target sequences are introduced or how they are introduced so that multiple genes are inactivated.

Claims 35-40, 56 and 57 are unclear in the recitation of 'promotes' since claim 32 requires that 'recombination between two six sites in eukaryotic cells is obtained'. The metes and bounds of promotes is unclear because claim 32 only results in recombination. It is unclear given the presence of the *six* sites and the addition of beta recombinase, how any other outcome besides recombination occurs.

Claims 41-44 are vague and confusing in the recitation of 'as an extrachromosomal DNA substrate' because the claims are directed to methods in transgenic animals, and so it is unclear how an extrachromosomal substrate would be present in the methods. Further, claims 49 and 50 are confusing because the activity of beta recombinase results in intramolecular recombination between two six sites, and given this activity, it is unclear how extrachomosomal DNA would be integrated into the genome.

Claim 52 is vague and confusing because independent claim 27 recites a method for mediating intramolecular recombination in eukaryotic cells, however claim 52 recites a method

for developing transgenic mammals. Further, claim 52 is incomplete, though it recites several method steps, the final step does not result in a transgenic cells.

Claims 58-60 are unclear and incomplete because even providing necessary cofactors, the method will not work unless the prokaryotic beta recombinase and *six* sites must be present in the eukaryotic cell. Beta recombinase does not exist in eukaryotic cells so it is unclear how supplying cell factors would promote beta recombinase activity.

Conclusion

No claim is allowed.

Claims 27, 28, 31-33, 35-50 and 52-60 are free of the are of record because the art fails to teach the use of beta recombinase in the generation of transgenic animals. Applicants were the first to describe the properties of beta recombinase as a member of the resolvase/invertase family of recombinases. While other recombinases (Cre and Flp) have been used in the art to generate transgenic animals, these recombinases are from the Int family of recombinases and do not require additional cofactors. Beta recombinase was isolated from a prokaryotic cell and requires the cofactor Hbsu to affect recombination between two *six* site target sequences. Applicants are the first to demonstrate that in the presence of the mammalian cofactor HMG1, beta recombinase is capable of generating an intramolecular recombination event between two *six* sites in mammalian cells, and thus, as other previously described recombinases, would be useful in the generation of transgenic mammals.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Joseph Woitach whose telephone number is (703)305-3732.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karen M. Hauda, can be reached at (703)305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Kay Pinkney whose telephone number is (703)306-3076.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center numbers are (703)308-4242 and (703)305-3014.

Joseph T. Woitach

Deborah Crouch

DEBORAH CROUCH
PRIMARY EXAMINER
GROUP 1800 1630